Environmental Health Perspectives
Vol. 25, pp. 125-128, 1978

Effect of Copper and Zinc Status on Susceptibility to Cadmium Intoxication

by I. Bremner* and J. K. Campbell*

The effects of dietary cadmium on copper and zinc metabolism in animals are described. Emphasis is given to situations involving chronic exposure to low levels of cadmium, to the identification of population groups most at risk, and to the protective effect of dietary supplementation with copper and zinc. The mechanism of the interaction between the metals and the involvement of metallothionein are discussed.

It is now widely recognized that the toxicity of metals cannot be considered without due regard being given to dietary composition and the nutritional state of the animal. Increased cadmium intake can cause alterations in the metabolism of copper and zinc in experimental animals and, conversely, the development of certain symptoms of cadmium toxicity may, on occasion, be prevented by dietary copper and zinc supplementation. For example, elevated cadmium intakes in rats, chicks and mice have resulted in increased mortality, poor growth, and anemia (*1, 2*). The growth rate was restored by zinc supplementation and the mortality and anemia reduced by increasing the copper intake. Supplementation with copper also prevented the degeneration of aortic elastin, presumably by restoring the activity of the copper-dependent enzyme lysyl oxidase. Clinical signs of zinc deficiency have been reported in poultry (*3*) fed on high cadmium diets, but were absent in animals in which the zinc intake had been increased.

The widespread occurrence of competitive antagonisms between metals has been explained in terms of their isomorphous replacement at particular sites in biological systems. This concept was of considerable value in furthering our understanding of the multiplicity of trace metal interactions, but it did not explain all facets of trace metal imbalance.

One difficulty was that the antagonistic effect of a metal was, on occasion, associated with increased concentrations of the agonist in tissues exhibiting signs of pathological change, rather than with the decreased concentrations that might be expected. For example, the testicular atrophy occurring in rats after cadmium injection was associated with a massive increase in zinc concentrations in the testes and yet was preventable by prior zinc injection (*4*).

Although many reports have been published illustrating the complexity of the interactions among cadmium, copper, and zinc, these have usually left unanswered the fundamental question of whether copper and zinc metabolism is likely to be disturbed at the level of exposure encountered by human and animal populations. Typical concentrations of cadmium in the human diet are about 0.05 mg/kg and may increase to about 1 mg/kg in contaminated areas. In many experimental studies to date, the concentrations of cadmium used have been greater than this by several orders of magnitude. Furthermore, the diets used have often been frankly deficient in both copper and zinc, although deficiency states of comparable severity are almost unknown in human populations. There is, however, an increasing realization that dietary zinc intakes may be only marginally adequate for normal demands and that copper deficiency may occur in special circumstances, as in cases of malnourishment in infants. In view of the occurrence of skeletal defects and cardiovascular lesions in both chronic cadmium toxicity and copper deficiency, there is a need for detailed assessment of the importance of the interactions between these metals. This can only be done by direct experimentation in animals, using realistic concentrations of cadmium, zinc, and copper. It must also be recognized that the effects of cadmium on copper and zinc metabolism may be more evident in certain population groups, because

* Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, United Kingdom.

of increased demands on body reserves of essential trace elements during periods of rapid growth, pregnancy, and lactation.

Concern has been expressed about the hazards of cadmium intoxication in animals grazing near industrial complexes where pastures may contain about 10 mg cadmium/kg. In studies designed to assess this danger, Mills and Dalgarno (*5*) found that copper metabolism was seriously disturbed in pregnant ewes, and more especially in their lambs, when they were fed diets containing only 3.5–12 mg cadmium/kg, even though their copper intake, 5 mg/kg, was apparently sufficient to meet normal requirements. Both liver and plasma copper concentrations were markedly reduced but the only clinical manifestation of copper deficiency was a deterioration in wool quality. Zinc metabolism was not seriously affected, despite a decrease in liver zinc contents. In agreement with the observations of Anke et al. (*6*), there appeared to be an efficient placental block against cadmium transport to the fetus, but there was nevertheless still a trend towards reduced copper accumulation in the newborn lamb. Little or no cadmium was accumulated by suckling animals, but newly weaned animals appeared particularly susceptible to cadmium following their introduction to solid food.

In contrast, no disturbances in copper metabolism were detected in pregnant ewes receiving 3 mg cadmium/kg and only 2.6 mg copper/kg, which was still sufficient to maintain normal growth and blood indices in the control animals (Campbell and Mills, unpublished data). However, the offspring of the cadmium-treated animals had lower birth weights, and there were signs of skeletal rarefaction and significant reductions in growth, plasma copper concentrations, and cytochrome oxidase activities of liver and duodenal mucosa when they were fed these diets (with 2.5 or 4.5 mg copper/kg) for several months thereafter. These effects were abolished by increasing the dietary zinc intake from 30 to 150 mg/kg. Surprisingly, this was associated with an increase in hepatic copper concentration, in contrast to the decrease noted by Bremner, Young, and Mills (*7*) in demonstrating a protective effect of zinc against copper toxicity in sheep. The effects of both cadmium and zinc on the growth of the lambs were abolished by increasing the dietary copper content to 15 mg/kg.

The inclusion of a range of dietary zinc concentrations in these experiments was inspired by the common occurrence of both cadmium and zinc in excessive amounts in contaminated pastures (*5*). A dietary zinc content of 750 mg/kg severely reduced food intake, growth, and copper status of the pregnant ewes. It also caused a high incidence of abortions and reduced the viability of the lambs. Increasing the dietary copper content prevented the decrease in plasma copper concentrations but had no effect on growth or on lamb survival, which indicates that these effects did not result from a conditioned copper deficiency. Instead it is probable that they resulted from the low food intake of the ewes or the accumulation of zinc in fetal kidneys and other tissues, with consequent renal damage similar to that reported in lambs on liquid diets with a high zinc content (*8*). It is noteworthy that adult sheep can tolerate zinc intakes of 400–1000 mg/kg with at most only slight effects on growth and food intake. It is apparent therefore that the pregnant or young liquid-fed animal is more susceptible to excessive zinc intakes. However, in the latter case, this may be a reflection of a more general phenomenon since the young of many species show increased absorption of several heavy metals compared with older animals.

Similar findings of decreased plasma ceruloplasmin and kidney copper concentrations and reduced cortical bone index have been made in rats fed a diet with as little as 1.5 mg cadmium (*9*, Campbell and Mills, unpublished data). These effects were exacerbated by cadmium intakes of up to 18 mg/kg, which reduced liver copper concentrations by about 50%. Plasma zinc concentrations were reduced, and liver and kidney zinc concentrations were increased. However, the changes in zinc content were relatively minor and, unlike the situation in sheep, there was no beneficial effect on increasing the zinc content of the diet. Instead, this increased the severity of the copper deficiency state, although it did reduce kidney cadmium concentrations. Indeed, dietary intakes of 300 and 1000 mg zinc/kg, even in the absence of cadmium supplements, had severe effects on copper metabolism.

The reductions in plasma and tissue copper concentrations caused by a cadmium intake of 6 mg/kg were prevented by increasing the copper intake, indicating that there was a direct effect of cadmium on copper metabolism.

This could arise from a reduction in copper absorption or alternatively a change in copper distribution within tissues and its displacement from functional sites. Van Campen (*10*) and Starcher (*11*) have claimed that cadmium inhibits ^{64}Cu absorption in rats and chicks, possibly by inhibition of copper-binding to a low molecular weight protein in the mucosal cytosol, which may be involved in the copper absorption process (*11*, *12*). Davies and Campbell (*13*) confirmed that cadmium inhibited copper absorption at a molar cadmium:copper ratio as low as 4:1 which was similar to that found sufficient to induce a copper deficiency state in rats (*9*).

However, in contrast with the earlier reports, they found that binding of ^{64}Cu to the intestinal mucosa was increased, even at a cadmium:copper ratio of 1:1. This was associated in part with the low molecular weight copper protein, the binding of ^{64}Cu to the protein being inversely proportional to the cadmium intake. It appears, therefore, that cadmium may block the exit of copper from mucosal cells, while exerting little inhibitory effect on its mucosal uptake. The lack of agreement as to the effects of cadmium on mucosal binding of copper may derive from the abnormally high cadmium:copper ratios used in the earlier studies (*10, 11*) or from the fact that Davies and Campbell (*13*) maintained their rats on the cadmium-supplemented diet for one week prior to dosing with ^{64}Cu, as this increased the concentrations of cadmium and zinc in the low molecular weight fraction.

The nature of the intestinal metal-binding proteins has yet to be established. It has been assumed by several groups that the cadmium protein is metallothionein, but, according to Evans and LeBlanc (*14*), the copper protein may have a different amino acid composition. However, similar claims have been made as to the identity of the analogous copper-protein in liver (*15*), yet Bremner and Young (*16*) successfully isolated (copper, zinc)-thioneins from the livers of copper-injected rats.

Many of the cadmium-induced changes in tissue copper and zinc distribution appear to result from increased incorporation of the other metals into metallothionein. For example, cadmium administration causes an increase in the amount of zinc in hepatic metallothionein and of copper in renal metallothionein (*17*). Although cadmium, zinc, and copper can all apparently induce synthesis of metallothionein, displacement of one metal by another and competition for binding sites on the protein may also occur. For example, cadmium has a greater binding affinity than zinc for binding sites, and high copper concentrations in ovine liver have resulted in displacement of both zinc and cadmium from metallothionein (Bremner, unpublished data). Furthermore, the zinc status of an animal has an important influence on the accumulation of copper-thioneins in liver and kidney (*18, 19*), possibly because of the decreased biological stability of the zinc-free copper-thioneins (*20*).

It is not known whether the accumulation of cadmium-thionein after chronic exposure to cadmium is influenced by zinc status, but it has been shown that the lethality (*21*) and development of testicular atrophy (*4*) and alterations in hepatic and pancreatic function (*22*) in acute cadmium toxicity are diminished by zinc administration. This has been attributed (*21*) to increased and more rapid incorporation of cadmium into metallothionein as a result of its prior induction by zinc (*23*). However, the importance of preinduced metallothioneins in the protection against acute cadmium toxicity has recently been disputed (*24*).

These conclusions do not necessarily apply to animals chronically exposed to cadmium where it can be argued that any displacement of cadmium from metallothionein will lead to greater expression of the toxicity of cadmium. For example, the conversion of vitamin D into the active 1,25-dihydroxycholecalciferol derivative in the kidney is inhibited by cadmium, but not by cadmium-thionein (*25*). It is possible that cadmium bound to other proteins might still inhibit the activation of vitamin D, with eventual development of skeletal lesions typical of cadmium toxicity.

It is interesting that the hydroxylation of vitamin D is due to a mixed function oxidase reaction and may therefore be a copper-dependent process (*26*). The occurrence of disturbances in vitamin D metabolism in cadmium-treated animals could therefore result from direct inhibition by cadmium of the hydroxylation or indirectly by induction of a copper deficiency state. These postulated effects of ω-hydroxylations may have wider implications in view of the reduction in cytochrome P-450 contents and inhibition of drug metabolizing enzymes in both cadmium-treated (*27*) and copper-deficient rats (*28*).

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